UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Reregistration of Tribufos (List B, Case 2145, Chemical 74801). Nature of the Residue in Cotton. Nature of the Residue in Poultry and in Ruminants. Storage Stability in Soil. Waiver Requests for Feeding Study and Animal Commodity Analytical Methods. DP Barcodes D169854 and D179581. MRID Nos. 42350009, 42350011, 42354503, 42350010, 42034502, 42350008, and 42350012. CBRS Nos. 8763 and 10031.

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Miles Inc. has submitted studies on the metabolism of radiolabeled tribufos in/on cotton (MRID 42350009), in lactating goats (MRID 42354503; 42350011), and in laying hens (MRID 42350010; 42034502) and on the stability of tribufos in soil (MRID 42350008). The registrant has also requested a waiver from the requirements for animal feeding studies and for an animal commodity enforcement analytical method (MRID 42350012).

Tribufos, or S,S,S-Tributyl phosphorotrithioate, $(CH_3CH_2CH_2CH_2S)_3PO$, is a cotton defoliant. It is used as a bottom defoliant to reduce or prevent losses from boll rot organisms (1.12 lbs. a.i./A), as a mix with the last insecticide application to accelerate the aging of cotton leaves, and as a total defoliant (1.9 lbs. a.i./A) about

Recycled/Recyclable
Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

4 - 7 days before total defoliation is desired (DEF 6, Emulsifiable Defoliant, 3125-282). Grazing restrictions are imposed.

In the Phase 4 Review (J. S. Smith, 08/30/90), it was noted that Mobay had pledged to generate new studies for GLN's 171-4(a) to 171-4(k/l).

Tolerances have been established for residues of tribufos in/on cottonseed (40 CFR §180.272) at 4.0 ppm and in/on cottonseed hulls (40 CFR §186.5800) at 6.0 ppm. Tolerances have also been established (40 CFR §180.272) for residues of tribufos in ruminant meat, meat byproducts, and fat, each at 0.02 ppm (N), and in milk at 0.002 ppm (N).

All studies were conducted by the Research and Development Department, Agricultural Division, Miles Inc., Stilwell, Kansas.

Conclusions

1. The nature of the residue study in cotton fulfills the requirements of GLN 171-4(a) for purposes of the reregistration of tribufos. The residue of concern consists of parent tribufos only. Solvent extraction removed > 80% the trr from foliage. Tribufos composed > 80% of the trr in foliage, and multiple polar metabolites comprised an additional 10%. Residue levels on the delinted seed were about 0.07 ppm from the 3.3X application. A combination of solvent extraction and mild acid hydrolysis removed about 50% of the trr from cottonseed. The extracted/hydrolyzed residues from seed were almost totally tribufos (50% trr). The residue not removed from seed (<0.04 ppm) was assumed to be bound.

2. The nature of the residue study in poultry and the nature of the residue study in ruminants (goats) fulfill the requirements of GLN 171-4(b) for purposes of the reregistration of tribufos.

a. In poultry, relatively low levels of radiolabeled residues were found in eggs and tissues from a study conducted at a 190X exaggerated feeding rate. The total radiolabeled residues in muscle and fat were about 0.5 ppm each. The trr in day 3 internal egg was 1.9 ppm, but only 0.3 ppm in day 2 egg. The maximum trr occurred in liver, 4.9 ppm. Solvent extraction and base hydrolysis released virtually all of the radiolabel from liver. Solvent extraction released 94% trr from fat, 85% trr from muscle, and 90% trr from eggs. About 65% trr was identified in muscle, and 77% was identified in eggs. The percentages identified in the other tissues were low: 21% in liver, and 20% in fat. However, evidence was presented that tribufos was extensively metabolized with the radiolabel being incorporated into natural products (malic acid, fatty acids, triglycerides). It is estimated that 24% - 50% of the total radiolabeled residues in eggs, liver, and fat were natural products. The major identified metabolite (exocon) was 3-hydroxybutylmethyl sulfone (HBM sulfone; 0% - 38% trr).

Tribufos was found only in eggs (4% trr, 0.08 ppm) and in fat (7% trr, 0.04 ppm). It is very improbable that tribufos will be found in poultry commodities from ingestion by hens of the defoliant at 1X - 10X anticipated dietary exposure levels. It is concluded that there is no residue of concern in poultry.

b. In ruminants, the radiolabeled residues ranged from 0.06 ppm in muscle to 3.5 ppm in liver from oral administration of ^{14}C-tribufos at a 5X exaggerated rate. The residue in milk may have plateaued at 0.16 ppm, but data variability make this a speculative conclusion. The following percentages of radiolabeled residues were released by solvent extractions: 74% milk; 71% fat; 12% liver; 49% muscle; 58% kidney. Additional residues were released from liver (88% trr), muscle (44% trr), and kidney (46% trr) by base hydrolysis (2N reflux) of the post-extraction solids.

In milk, the major metabolites were 3-hydroxybutylmethyl sulfone (HBM sulfone, 33% trr) and tribufos (5% trr). Only 54% trr was identified. However, this included 12% as fatty acids, showing degradation of tribufos and incorporation into natural products. The major metabolites in muscle were HBM sulfone (44% trr) and butyl mercaptan (13% - 29% trr). Tribufos was 0.5% trr. The major metabolite in liver and kidney was butyl mercaptan (15% - 35% trr and 5% - 25% trr, respectively), but very little of the radiolabeled residue was identified (see below). Butyl mercaptan was released by base hydrolysis and use of DTT to cleave disulfide linkages. Therefore, the butyl mercaptan probably represents radiolabeled material bound to proteins. The major metabolite in fat was tribufos (31% trr). The presence of radiolabeled fatty acids (7% trr) in fat indicated degradation of tribufos and incorporation into natural products.

The metabolites in liver were only partially identified. Because various solvents failed to extract the radiolabeled residue (< 20% trr), the registrant used a characterization scheme based on the distribution of the radiolabel in trichloroacetic acid (solubles, 10% trr), chloroform/ethanol (lipid, 8% trr), perchloric acid (nucleic acids, 1% trr), and residual solid (proteins, 81%). The registrant failed to identify the residue released (88% trr) by base hydrolysis of the post-solvent extracted liver.

c. For both poultry and ruminant studies, adequate storage stability data were presented. The extractable metabolites were stable for the time periods involved in the nature of the residue studies.

3. The storage stability of tribufos in soil is not within the purview of CBRS. This submission was not reviewed.

4a. A feeding study (171-4(j)) is required for ruminants. The nature of the residue study clearly shows the potential for residue

accumulation at 1X dietary exposure, and the residue of concern (tribufos) was found in milk and fat. The waiver request from a ruminant feeding study should be denied. Lactating dairy cows should be fed tribufos at 1X, 3X, and 10X levels for 30 days or longer if residues have not plateaued in milk.

4b. A feeding study (171-4(j)) is not required for poultry, and the waiver request should be granted. The nature of the residue study conducted at a 190X exaggerated rate revealed tribufos at low levels (<0.1 ppm) in eggs and fat only. A detectable level of tribufos at a 1X feeding level is unlikely. The 1X level is calculated based on established tolerances for cottonseed (4.0 ppm) and cottonseed hulls (6.0 ppm). While these tolerances will be reevaluated based on new field trials and a processing study pledged by the registrant, the nature of the residue in cotton study (0.07 ppm trr from 3.3X application) indicates that the existing tolerances are conservative.

5. The registrant's request to delete the existing tolerances for residues of tribufos in meat (0.02 ppm) and milk (0.002 ppm) is not warranted. These tolerances will be reevaluated after receipt of an acceptable ruminant feeding study (171-4(j)). Likewise, the existing tolerances for cottonseed and cottonseed processed commodities will be reevaluated after receipt of the pledged cotton field trial and processing studies.

6. An analytical enforcement method (GLN 171-4(d)) will be required for the determination of residues of tribufos in ruminant milk, meat, and fat. The request for a waiver should be denied. The method must be tested with radiolabeled samples from the nature of the residue in ruminants study. The final method must be validated by an independent laboratory before submission to the Agency.

Recommendation

The requirements for determination of the nature of the residue in/on cottonseed and in ruminants and poultry for the defoliant tribufos have been fulfilled. No additional data are required for 171-4(a) and 171-4(b).

CBRS recommends that the requirement for a feeding study for poultry be waived (171-4(j)). CBRS further recommends that the request for a waiver from the requirement for a feeding study for ruminants be denied. A feeding study is required for ruminants, and should be conducted as indicated in Conclusion no. 4a.

CBRS further recommends that no changes be made in the existing tolerances for residue of tribufos until the required feeding study, field trial studies, and processing study are completed and reviewed.

CBRS further recommends that the storage stability study for tribufos in soil be redirected to the appropriate Division, possibly EFED.

Detailed Considerations

Nature of the Residue in Cotton

Field Phase

Cotton plants were grown in sandy loam soil in 5 gallon plastic buckets in a greenhouse. Ten, 11-week old plants were placed in a plastic lined chamber (5 ft X 10 ft) in the greenhouse. At least 50% of the bolls were open on each plant. Sunlight was supplemented by 450-watt high pressure sodium vapor lamps for 15 hours each day. Average relative humidity during the test period (01/90 - 03/90) was 27.9%, maximum 74% and minimum 7%. The average temperature was 27.8° C, maximum 50.5° C and minimum 21.7° C. A small hand-held sprayer was used to apply a [^{14}C]-tribufos spray emulsion to the ten plants on 02/27/90. The application rate was equivalent to 6.2 lbs. a.i./acre, or 3.3X the maximum label rate of 1.9 lbs. a.i./acre (DEF 6 Emulsifiable Defoliant, Reg. No. 3125-282).

The test compound was [n-butylthio-1-^{14}C] tribufos with a specific activity of 20.4 mCi/mmole, or 144000dpm/μg, and a radiochemical purity (tlc) >97%. The ^{14}C-label was on the CH^2 attached to the S of one of the butylthio groups (see Figure 1). The test formulation consisted of 283.5 mg of the ^{14}C-tribufos and 586 mg of unlabeled tribufos in 355 mg of blank formulation media (such as used for DEF 6). This concentrate was mixed with 104.6 ml of deionized water, and the resulting mixture was sonicated to form an emulsion. The final specific activity of the emulsion was 6.8 mCi/mole, or 46563 dpm/μg.

Zero time samples were taken two hours after the application. One open boll and two leaves were taken from each plant. Three day posttreatment samples were taken on 03/02/90. One open boll and four dry leaves were taken from each plant. The final harvest was 9 days posttreatment (03/08/90). All dry leaves were taken and pooled. All open bolls were collected and pooled. Samples were stored at -20° C.

Analysis

The 0-day and 3-day posttreatment foliages (10 g samples) were rinsed with acetonitrile (2 or 3 X 100 ml). The rinsed leaf samples were homogenized with acetonitrile (2 X 50 - 100 ml), and the homogenates were filtered. Aliquots of the various acetonitrile fractions, both before and after concentration of the fractions, were radioassayed. The concentrates were analyzed by

tlc. The residual leaf solids were air-dried and radioassayed.

The final harvest cotton foliage (9-day posttreatment) was extracted by several different procedures. In procedure no. 1, an aliquot (5.6 g) of pulverized foliage was sequentially extracted (Polytron homogenizer) with methanol and water. The methanol extract was subjected to gel permeation chromatography (gpc, Bio-Beads SX-3, ABC 1002A) and the appropriate chloroform eluates analyzed by tlc and gc/ms. The water extract was lyophilized and dissolved in methanol. Various aliquots of the methanol and water extract were analyzed by tlc and hplc. Procedure no. 2 used chloroform, rather than methanol, for the extraction. Procedure no. 3 used chloroform, followed by methanol, for the extractions. The methanol extract was subjected to gpc. There was no aqueous extraction. Procedure no. 4 was similar to no. 3, but the methanol extract was resolved by hplc (Econosil C-18), and collected fractions were analyzed by hplc and tlc. For all procedures, appropriate fractions were radioassayed.

The cotton (lint plus seed) was picked by hand from the bolls, and the seeds were separated from the lint by hand. The seeds were delinted by dipping in 12 M sulfuric acid for 2 - 3 minutes. The delinted seeds were water-washed, air-dried, and pulverized with liquid nitrogen. The pulverized samples were combusted and radioassayed.

Pulverized seeds were extracted by three different procedures. In procedure no. 1, a sample (20.7 g) was extracted sequentially in a Polytron homogenizer with hexane, acetonitrile, and water. The hexane extract was partitioned with acetonitrile. Residual solids were stirred with 1 N HCl for 4 - 5 hours at room temperature. The mixture was filtered, and both the solid and acid solution were radioassayed. Procedure no. 2 (11.8 g sample) differed in that a water extraction step was not used and the hexane extracts were not partitioned with acetonitrile. In procedure no. 3, an aliquot (11.8 g) of pulverized seeds was Soxhlet extracted overnight with hexane (300 ml). The extracted seeds and hexane extract were combined and refluxed for three hours. The mixture was filtered and the refluxing procedure was repeated several times (300 ml hexane; 400 ml hexane; 400 ml acetonitrile). The combined hexane extracts and the acetonitrile extract were individually concentrated *in vacuo*. Appropriate fractions were radioassayed.

The hexane extracts of pulverized seed from the three procedures were combined and subjected to gpc. Twenty-three fractions were collected and radioassayed. Fractions 13 - 18 were combined and analyzed by tlc. Bands were scraped, and the silica gel was extracted with ethyl acetate. The extract was analyzed by tlc and gc/ms.

Normal phase tlc was conducted with pre-coated silica gel plates with a fluorescent indicator. The mobile phase was hexane/acetone

(9/1, v/v). The reversed phase tlc was conducted with pre-coated KC18-F plates with an acetonitrile/methanol/0.5 M NaCl mobile phase (2/1/1). Radioactivity zones were measured with a Raytest Radio-TLC Analyzer. Non-labeled compounds were detected with uv, or the plates were exposed to iodine vapors.

The hplc analyses were conducted on an Econosil C-18 analytical column with uv (254 nm) and solid scintillation detectors. A linear gradient elution was used, going from 50% B to 70% B in 15 minutes, 70% B to 90% B in 20 minutes, and holding at 90% B for 5 minutes. Solvent system A was water/acetic acid (99.9/0.1), and solvent system B was acetonitrile/tetrahydrofuran (85/15).

The reference standards for both tlc and hplc are shown in Figure 1.

The gc/ms analyses were conducted in both the electron impact and chemical ionization modes. Ammonia was used as the ionization gas for the latter. The column was a 0.32 mm i.d.. X 50 m DD-5 (Hewlett-Packard) interfaced directly with the ms. Injection was splitless. Example chromatograms were provided.

Results

The radioactivity levels found on the intact plant parts are summarized in Table 1. The distribution of the radioactivity among the extracts and residual solids from the workup of the cotton foliage and seed is given in Table 2. Adequate raw data were provided to verify the calculations and results.

Table 1: Radioactivity Levels in Cotton Plant Parts After Treatment With ^{14}C-Tribufos at 6.2 Lbs. A.I./Acre

Matrix	Posttreatment Days	Tribufos Equivalents (ppm)
Seed (Delinted)	0	0.01
	3	0.07
	9	0.07[1]
Foliage	0	92.5[2]
	3	253[2,3]
	9	175[2]

[1] Sum of results for extracts and residual solid. The registrant indicates that the seed prior to extraction was radioassayed, and calculations of expected radioactivity levels (Appendix 12) suggest that this is the case. Data not explicitly given.

[2] Sum of results for extracts and residual solid. The registrant indicates that the foliage prior to extraction was radioassayed, and calculations of expected radioactivity levels (Appendix 10) suggest that this is the case. Data not explicitly given.

[3] Increase (from day 0) attributed to loss of water from the foliage.

Table 2: Distribution of Radioactivity in the Washes, Extracts, and Hydrolysates of ^{14}C-Tribufos-Treated Cotton Plants

Matrix	Postharvest Days	Distribution of Total Radioactivity[1] (%)				
		Solvent Wash	Organic Solvent Extract	Aqueous Extract	1 N HCl Hydrolysate	Residual Solid
Foliage	0	74	21	-	-	5
	3	39	41	-	-	17
	9	-	90[2]	4.5[3]	-	8[4]
Seed	9	-	47[5]	-	18[6]	41[7]

[1] Distributions are based on the concentration found in a given extract or other fraction divided by the sum of all concentrations. Results are effectively normalized and would not reflect losses in radioactivity during the procedures. Data for 9-day foliage and 9-day seed indicate recoveries of 93% - 100% and 86% - 103%, respectively, for these particular matrices. Averaging of results within a particular extract gives total results different from 100% in this table.
[2] Average of 4 extraction procedures (94% no.1; 82% no. 2; 77% + 15% no.3; 66% + 26% no. 4).
[3] 2% procedure no.1; 7% procedure no. 2.
[4] Average of procedure nos. 1 - 4: 4%, 11%, 8%, 8%.
[5] Average of procedure nos. 1 - 3: 27% + 14%; 30% + 10%; 57% + 4%.
[6] Average of procedure nos. 1 and 2: 14; 21.
[7] Average of procedure nos. 1 - 3: 46; 39; 39.

Approximately 90% of the total radioactive residue was released from final foliage by a series of organic solvent extractions, and less than 10% trr remained in the postextraction solid. About 40% of the trr was released from the final harvest seed by organic solvents, and an additional 14% - 21% was released by mild acid hydrolysis. About 40% remained in the postextraction solid.

The acetonitrile washes and extracts from 0 day and 3 day posttreatment foliage were analyzed by tlc. The tlc radiochromatograms showed one major peak and a very small amount of origin material. The peak corresponds (R_f) to tribufos. The tlc was performed on reversed phase KC18-F and normal phase silica gel plates. The registrant reports that the peak corresponds to 78% of the trr. No data are presented, but the radiochromatograms clearly show that the tribufos peak is >90% of the radioactivity in the acetonitrile fraction, and 90% X 80% trr, the percentage in the combined acetonitrile fractions of 3 day foliage, yields 72%. For 0 day foliage the value is 85%. No additional analyses are reported for the 0 and 3 day posttreatment foliage, although hplc analyses are mentioned in the text.

The methanol and aqueous extracts from procedure no. 1 for final harvest foliage were analyzed by silica gel tlc and by hplc. The tlc radiochromatogram of the methanol extract (94% trr) shows tribufos as the main peak, along with small amounts of origin material and a peak just after the origin. The aqueous extract (2% trr) tlc shows only origin material. The hplc chromatogram (radiochemical detector) of the methanol extract shows only tribufos. The chromatogram of the aqueous extract shows no responses above background. The tlc radiochromatograms from gpc clean-up of the methanol extract shows only tribufos. No integration data were given with any of the chromatograms.

The tlc chromatograms of the organic extracts for procedure no. 2, no. 3, and no. 4 showed the same results as those for procedure no. 1. The aqueous extract (procedure no. 2, 7% trr) showed four poorly resolved peak regions on the reversed phase tlc radiochromatogram, with one minor region corresponding to tribufos.

The silica gel tlc radiochromatograms of gpc fractions 13 through 20 from the methanol extract, procedure no. 3, showed primarily (about 90%) tribufos. The radiochromatograms of fractions 8 through 12 showed primarily (about 95%) origin material. The reversed phase radiochromatograms of the same fractions 8 through 12 showed origin material plus five lesser peak regions, none of which corresponded to tribufos. The peaks totaled 2% trr, each <0.1 to 0.4% trr. The methanol extract from procedure no. 4 (26% trr, 45 ppm) showed minor peaks (reversed phase tlc radiochromatogram) in addition to tribufos. Peak isolation from this extract was attempted by hplc. The tlc radiochromatograms of the two collected fractions showed tribufos in the second hplc fraction and a mixture of about 6 compounds in the first fraction. The registrant reports that these 6 compounds account for no more than 3% of the total radioactive residue (5 ppm), each 0.1% to 1%. No integration or area data were provided for any of the chromatograms.

The electron impact mass spectrum of the major component in the methanol extract (procedure no. 1, 94% trr, 166 ppm) matched the spectrum of tribufos.

The registrant concludes that tribufos accounts for 79% - 89% of the foliage trr, depending on the extraction method, that silica gel tlc origin material accounts for 0% - 11%, and that other polar residues account for 1% - 3% trr. The exact values cannot be verified, because no integration data were presented for any of the tlc and hplc chromatograms. Visually, however, it is clear that tribufos is the major component, estimated to be >80% trr.

The hexane extract of seed (procedure no. 3, 57% trr, 0.04 ppm) yielded a silica gel radiochromatogram with dispersed radioactivity and possibly two peak regions. The silica gel radiochromatogram of the gpc fractions 14 to 18 from the hexane extract shows a minor peak after the origin and the tribufos peak. The latter is visually estimated to be >80% of the radioactivity, or 80% X 57%, 45% trr. The hplc confirmed the tribufos identification. The electron impact and chemical ionization mass spectra of the isolated compound match those of tribufos.

The registrant concludes that tribufos remains virtually unmetabolized on foliage and that about 50% is unmetabolized in/on cottonseed. The remainder (<0.04 ppm) in cottonseed is bound or incorporated into natural constituents.

Storage Stability

A concurrent storage stability study was conducted. Fresh, untreated cotton leaves and seeds were ground separately, and 10 g subsamples were spiked with 1.0 ml portions of a methanol solution containing 10.36 μg/ml (1491361 dpm) of ^{14}C-tribufos. The methanol

was allowed to evaporate, and the spiked samples were stored in jars with lids in a walk-in freezer next to the test samples (-24° C). Samples were extracted with acetonitrile and analyzed (lsc, tlc, hplc) in triplicate on day 0 (03/29/90) and 41 days, 90 days, and 762 days posttreatment. The results are summarized in Table 3. No loss of tribufos occurred over the 762 day period. The storage interval for the metabolism samples was a maximum of 210 days.

Table 3: Concurrent Storage Stability of ^{14}C-Tribufos in Frozen (-24° C) Cotton Foliage and Seeds[1]

Matrix	Interval (days)	Radioactivity Extracted[2] (ppm)	Tribufos[3] (ppm)	Recovery[4] (%)
Foliage	0	0.84	0.84	82
	41	1.00	1.00	97
	90	0.95	0.95	92
	762	0.99	0.99	96
Seeds	0	0.84	0.84	82
	41	1.01	1.01	98
	90	0.98	0.98	95
	762	0.94	0.94	91

[1] Fortified at 1.03 ppm ^{14}C-Tribufos.
[2] Tribufos equivalents.
[3] Hplc analysis of the acetonitrile extract.
[4] Based on fortification concentration.

Nature of the Residue in Poultry

In-Life Phase

Two test substances were used. The first was 1-^{14}C-tribufos. The ^{14}C-label was on the CH_2 attached to the S of one of the butylthio groups. The specific activity was 143,991 dpm/μg or 20.4 mCi/mmol, and the radiochemical purity was > 98%. No natural abundance tribufos was used in preparing the test formulation. The ^{14}C-tribufos was dissolved in methylene chloride (144 mg/2.4 ml), and 93 μl aliquots were placed in each capsule. The solution was radioassayed and analyzed by hplc.

The second test substance was ^{35}S-tribufos, labeled on one of the three sulfurs. The specific activity was 182,118 dpm/μg, or 25.8 mCi/mmol, and the radiochemical purity was > 99%. The ^{35}C-tribufos was dissolved in acetonitrile, and 204 μl aliquots were placed in gelatin capsules. The solution was radioassayed and analyzed by

hplc.

Ten laying hens were acclimated for four months prior to the three-day study in a climate-controlled room (25° C, 70% relative humidity, 16 hrs.light and 8 hrs. dark cycle). Feed and water were given *ad libitum*. Four of the chickens were dosed daily with ^{35}S-tribufos at a nominal rate of 4 mg/kg body weight/day, and the remaining six were dosed daily with ^{14}C-tribufos at a nominal rate of 4 mg/kg body weight/day. There were no control chickens (0X). The tribufos was administered orally in gelatin capsules containing lactose and the labeled tribufos. Eggs were collected each day before dosing, and all eggs from a given day were pooled. The chickens were sacrificed four hours after the final dose. The following samples were taken: composite muscle, composite fat, liver, and internal eggs. Samples were frozen (-20° C) and macerated the following day with liquid nitrogen in a Tissuemizer. The resulting powders were stored frozen, and all analyses were completed within 30 days of the sacrifice.

The chickens in the ^{14}C-tribufos group had an average weight of 1.50 kg, range 1.344 kg - 1.640 kg. The chickens in the ^{35}S-tribufos group had an average weight of 1.58 kg, range 1.577 kg - 1.804 kg. Based on the 4 ppm tolerance for tribufos in/on cottonseed, on 8% of the diet of laying chickens being derived from cottonseed processed commodities (Subdivision O, Table 2), on consumption of 100 g of feed per day per chicken (*Feeds and Nutrition, Ensminger Publishing Co.), and on an average chicken weight of 1.50 kg or 1.58 kg, the 4 ppm dose represents a 190X exaggerated feeding rate:*

Theoretical Average Daily Dose-
[(0.08) X 4 μg tribufos/g feed X (100 g feed/day/chicken)] / 1500 g body weight/chicken = 0.021 μg tribufos/day/g body weight.

Actual Dose Administered-
(126 mg ^{14}C-tribufos/21 capsules) / 1.50 kg = 4 μg ^{14}C-tribufos/g body weight/day.
Range 3.78 ppm - 4.46 ppm. Equivalent to 60 ppm tribufos in the feed.

(75.84 g ^{35}S-tribufos/12 capsules / 1.58 kg = 4 μg/g ^{35}S-tribufos/g body weight/day.
Range 3.50 ppm - 4.85 ppm. Equivalent to 63 ppm tribufos in the feed.

Exaggeration-
4 / 0.021 = 190.

Analysis

Extraction and Radioassay

The liver (77 g) of ^{14}C-tribufos treated hens was extracted repeatedly with methanol in a Tissuemizer. The liver of ^{35}S-tribufos treated hens was extracted repeatedly with water/methanol (50/50) in a Tissuemizer. Combined filtrates from each extraction were concentrated by rotary evaporation *in vacuo* at room temperature. The residual solids were air-dried and stored at -20° C.

A portion of the liver methanol extract from the ^{14}C-tribufos treated hens was concentrated and the resulting precipitate was treated with sodium borohydride in methanol. The methanol from the $NaBH_4$ reduction was combined with the liver methanol extract and hydrolyzed with 6 N HCl (90° C, sealed vial, overnight). The solution was neutralized and extracted with ethyl acetate.

A portion of the liver post extraction solids was dissolved in 6 N HCl and was heated at 40° C for 24 hours under a condenser. The solution was then distilled in an attempt to capture any volatile compounds, such as thiols or small chain fatty acids. The distillate was extracted with methylene chloride. A small portion of the methylene chloride extract was washed with 0.1 N NaOH to determine the presence of organic acids. The residual liquid in the distillation apparatus was extracted with methylene chloride at both acidic and basic pH's.

Another portion of the liver post extraction solids was refluxed in 2 N KOH for 4 hours. The hydrolysate pH was adjusted to 8, and the solution was treated with dithiothreitol (2 days, ambient) after degassing with nitrogen. Dithiothreitol would release any butyl mercaptan (BUSH) that might have formed disulfide bonds in the biological matrix. The solution was extracted with chloroform. The chloroform extract was treated with m-chloroperoxybenzoic acid to oxidize any BUSH to the less volatile butane sulfonic acid.

The methanol extraction procedure for composite muscle (349 g) from ^{14}C-tribufos treated hens was similar to that for liver. For ^{35}S-tribufos treated hens, the water/methanol extract of composite muscle (203 g) was partitioned with hexane, ethyl acetate, and chloroform.

Solids from the concentrated methanol extract of muscle from ^{14}C-tribufos treated hens were treated with sodium borohydride in methanol. The methanol from the $NaBH_4$ treatment was combined with the muscle methanol extract and hydrolyzed with 6 N HCl (reflux, overnight). The mixture was neutralized and extracted with ethyl acetate.

The internal egg sample (203 g) from ^{14}C-tribufos treated hens was extracted with methanol (1 X 300ml, 5 X 250 ml), followed by chloroform/methanol (3 X 500 ml, 2/1). The methanol extracts were combined and concentrated. The chloroform/methanol extracts were combined and concentrated. A similar procedure was used with the internal egg sample (96.6 g) from the ^{35}S-tribufos treated hens, but the methanol extraction (1 X 250 ml, 5 X 250 ml) and the chloroform/methanol extraction (2 X 250 ml) were followed by sequential extraction of the solid with methanol/water (3 X 500 ml, 50/50), water (1 X 500 ml), 1 N HCl (1 X 500 ml), 100 mM EDTA (1 X 500 ml). All aqueous extracts were combined.

Composite fat from ^{14}C-tribufos treated hens was extracted sequentially with methanol (2 X 250 ml) and hexane (3 X 250 ml). The combined methanol extract was partitioned with hexane, and the combined hexane extract was partitioned with methanol. The hexane extracts formed a viscous gel upon evaporation of the solvent. The gel was partitioned with acetonitrile, and the acetonitrile extract was added to the methanol fraction. The acetonitrile/methanol extract was evaporated (30° - 40° C), and acetonitrile-saturated hexane was added. The hexane fraction was partitioned with acetonitrile. The acetonitrile fraction was chromatographed on silica gel, stepping from 100% chloroform to 100% methanol. The same procedure was used for fat from ^{35}S-tribufos treated hens.

Excreta was removed daily, composited, and frozen (-20° C). The excreta from ^{14}C-tribufos dosed hens was extracted with methanol, and that from ^{35}S-tribufos dosed hens was extracted with methanol/water (3/1). Extracts were analyzed by hplc and tlc.

In addition to radiolabeled tribufos, 14 radiolabeled standards were used for hplc and tlc identification of the metabolites, as given in Figure 2.

Six different hplc systems (A - F) were used. System A consisted of a C-18 column and varying proportions of 0.1% aqueous acetic acid and acetonitrile at a flow rate of 1.5 ml/min. It was used for initial analysis and fractionation of samples. System B consisted of an Ultrasphere ODS 5 μm column and varying proportions of 0.1% aqueous acetic acid and methanol at a flow rate of 1.5 ml/min. Systems C and D consisted of a MicroPak silica 10 μm column and varying proportions of hexane and isopropanol. System E used the MicroPak column but varying proportions of hexane and 2% aqueous isopropanol. System F used the Ultrasphere column (system B) with 5 mM Q5 ion-pairing reagent in water and methanol. A variable wavelength uv detector and a Ramona radioactivity monitor were used with all systems.

The tlc work was conducted on precoated silica gel 60 F-254 plates. Five different solvent systems were utilized. Low levels of radioactivity on the plates was determined by scraping and radioassaying methanol extracts of the scrapings. Distribution of high levels of radioactivity was accomplished with a Radio-TLC Analyzer, including integration software. Non-radioactive peaks were visualized with iodine.

Mass spectrometry work was conducted on three systems, a capillary gc (15 m X 0.25 mm methyl silicon column) with an electron impact quadrupole mass spectrometer, a hplc (4.6 mm X 4.5 cm Ultrasphere ODS 5 μm reverse phase column) coupled to a thermospray mass spectrometer, and positive and negative chemical ionization mass spectrometer with direct insertion probe or capillary gc.

Results

The radioactivity levels found in the tissues and eggs are summarized in Table 4. All raw data needed to verify the results were provided.

Table 4: Concentrations of ^{14}C-Tribufos and ^{35}S-Tribufos in Hen Tissues and Eggs Resulting from the Oral Administration of 4 μg Radiolabeled Tribufos per Gram of Body Weight per Day[1]

Matrix	^{14}C-Tribufos (ppm)	^{35}S-Tribufos (ppm)
Liver	4.899	3.717
Fat	0.501	0.309
Muscle	0.557	0.510
Egg- Day1	0.039	0.048
Egg- Day 2	0.281	0.352
Egg- Internal[2]	1.872	0.848

[1] ^{14}C-Tribufos administered to 6 hens and ^{35}S-tribufos administered to 4 hens.
[2] Insufficient data to ascertain if residues plateaued in eggs.

If it is assumed that the tribufos S is not utilized in the synthesis of biochemical structures, then the extent of incorporation of tribufos degradation products into natural products may be estimated as the difference in radioactivity concentrations between the ^{14}C-tribufos and ^{35}S-tribufos for a given tissue and extraction procedure. The registrant notes that this estimate will understate the actual amount of incorporation to the extent that ^{35}S is utilized. The registrant notes that incorporation of the radiolabeled sulfur could occur through inorganic ^{35}S sulfate to yield such products as complex lipids, phenols, hydroxysteroids, and glycosaminoglycans. The distributions from the initial extractions are given in Table 5.

Table 5: Distribution of Radiolabeled Residue in the Initial Extracts of Poultry Matrices

Matrix	Fraction	^{14}C Residue		^{35}S Residue		Natural Product[1]	
		ppm	% TRR	ppm	% TRR	ppm	%TRR
Liver	Total	4.899	100	3.717	100	1.182	24
	Methanol and Methanol/Water	2.738	56	2.591	70	0.146	3
	Solids	2.161	44	1.104	30	1.057	22
	Acid Hydrolysis[4]/Water Extract	1.066	22	-	-	-	-
	Acid Hydrolysis/Methylene Chloride Extract	0.223	4	-	-	-	-
	Acid Hydrolysis/Volatile	0.443	9	-	-	-	-
	Acid Hydrolysis/Solid	1.289	26	-	-	-	-
	Base Hydrolysis[4]/Water Extract	1.274	26	-	-	-	-
	Base Hydrolysis/ Chloroform Extract	0.886	18	-	-	-	-
Fat	Total	0.501	100	0.309	100	0.192	38
	Hexane	0.281	56	<0.006	< 2	0.275	55
	Acetonitrile	0.184	37	0.271	83	-0.090[2]	-
	Solids	0.031	6.2	0.048	16	-	-
Muscle	Total	0.557	100	0.510	100	0.047	9
	Methanol and Methanol/Water	0.471	84	0.432	85	0.039	8
	Solids	0.085	15	0.078	15	0.007	1
Internal Eggs	Total	1.872	100	0.848	100	1.024	55
	Methanol	0.733	39	0.242	28	0.491	26
	Chloroform/Methanol	0.945	50	0.003	0.4	0.942	50
	Solids	0.193	10	0.603	71	-0.410[3]	-

[1] ppm = (^{14}C Residue - ^{35}S Residue). % TRR = [(^{14}C Residue - ^{35}S Residue) / (^{14}C Residue)] X 100.
[2] Registrant speculates that the excess sulfur is the result of natural products such as sulfated lipids.
[3] Registrant speculates that the excess sulfur is the result of the deposition of inorganic sulfate, Ca_2SO_4, in the egg.
[4] Acid and base hydrolyses were each conducted on a portion of the postextraction solid, i.e., the hydrolyses were not sequential.

Liver

The methanol extract (56% trr) was analyzed by hplc, system A. The chromatogram was complex and consisted of at least 13 peaks, LM1 - LM13, from 0 - 30 minutes. LM1, at 0^+ minutes, was the largest component, 17% TRR or 0.843 ppm. The remaining components were 2% - 5% TRR, or 0.111 ppm - 0.228 ppm. None of the peaks corresponded to tribufos. LM1 was more polar than any of the standards and may have been a combination of several compounds. Attempts at derivatization of LM1 failed. The actual integration data were not presented. This fraction was acid hydrolyzed (6N HCL, 90° C) and extracted (ethyl acetate) before attempting further characterization.

The hydrolysis yielded an ethyl acetate fraction, 11% TRR or 0.558 ppm, a water soluble fraction, 39% TRR or 1.921 ppm, and residual solid, 5% TRR or 0.260 ppm. The water soluble fraction was analyzed by hplc, System B, and ten peaks were noted, LMHA1 - LMHA10, <1% TRR to 12% TRR. Two compounds, comprising 31% of the water soluble fraction, were identified. LMHA1, 9% TRR or 0.465 ppm, was isolated and identified (after methylation) as malic acid by hplc retention time. The same metabolite was found in excreta. In the latter case, the metabolite was identified by gc/ms, both ei and ci.

Metabolite LMH4A (3% TRR, 0.161 ppm) had a retention time that matched 3-hydroxybutylmethyl sulfone (HBM sulfone). The compound was purified by HPLC and acetylated. The hplc retention time of acetylated LMH4A and the gc/ms spectrum matched those of authentic HBM sulfone acetate.

The ethyl acetate extract (11% TRR) of the acid hydrolysis of the liver methanol extract contained four components (HPLC, system B, 8 minutes to 22 minutes), LMH01 to LMH04, 1% to 7% TRR. Two compounds were identified. LMH01, 7% TRR or 0.345 ppm, was isolated and identified (hplc, gc/ms) after acetylation as 3-hydroxybutylmethyl sulfone (HBM sulfone). A second compound, LHM04, 2% TRR or 0.086 ppm, was identified by hplc retention time only as butylmethyl sulfone (BM sulfone).

The distillate (volatiles) from the acid hydrolysis of liver postextraction solid (44% TRR, 2.161 ppm) contained 9% TRR, or 0.429 ppm. The hplc chromatogram (system B) showed about 9 regions (LDM1 to LDM9, <1% to 2%, 0.021 to 0.072 ppm) of radioactivity response. Silica gel tlc indicated the absence of tribufos. The radiochromatogram for the tlc was not provided. No additional characterization was reported.

The residual aqueous layer from the acid hydrolysis of liver postextraction solid contained 26% TRR, or 1.289 ppm. Partition with methylene chloride at pH < 1 and at pH 12 did not remove

significant amounts of radiolabeled residue, 1% at acid pH and 3% at alkaline pH. About 9% TRR was unaccounted in the acid hydrolysis.

The aqueous layer (26% TRR, 1.274 ppm) from the base hydrolysis and dithiothreitol reduction of the liver postextraction solids and after partitioning with chloroform could not be analyzed. Adjustment of pH precipitated the dissolved biological matrix. Adequate concentration for hplc analysis could not be achieved. The tlc analysis (system E) showed two extremely polar components.

The chloroform fraction (18% TRR, 0.886 ppm) from the base hydrolysis was oxidized with MCPA with the aim of oxidizing BUSH to butane sulfonic acid. After oxidation, the radioactive residue was partitioned between ether and water. Butane sulfonic acid would be found in the aqueous fraction. However, most of the radioactivity remained in the ether layer (13%, 0.637 ppm). The aqueous layer contained 5% TRR, 0.249 ppm. The ether layer contained three metabolites, ranging from <1% to 10% TRR (0.025 ppm to 0.477 ppm). All were more polar (tlc) than either tribufos or dibutyl disulfide (DBDS). No radiochromatograms were supplied.

The aqueous layer was shown not to contain butane sulfonic acid (tlc, hplc). Hplc analysis with an ion-pairing agent for acids, Q5, did show that most of the radioactivity was an acid.

The combined aqueous and methanol/water extracts (70% TRR, 2.591 ppm) from the ^{35}S-tribufos chicken livers showed 14 metabolites (hplc, system A), LSM1 to LSM14, ranging from 1% TRR to 12% TRR, 0.047 ppm to 0.415 ppm. The more polar metabolites comprised most of the residue, LSM1 at 11% and LSM2 at 12%. No further characterization was reported.

Fat

The hexane fraction (37% TRR, 0.184 ppm) was complicated by the presence of triglycerides, according to the registrant. No details were provided. Hplc (System D) showed the presence of three metabolites, FHM1 (18% trr, 0.091 ppm), FMH2 (6% trr, 0.030 ppm), and FMH3 (13% trr, 0.063 ppm). FMH3 was not a distinct peak. Metabolite FHM2 had a retention time similar to that of tribufos, but triglycerides also eluted at this retention time. No additional characterization was made.

The combined acetonitrile/methanol extract (57% trr, 0.286 ppm) was partitioned with acetonitrile and hexane to transfer any potential phospholipids to the hexane fraction. The hexane contained 20% trr, and the acetonitrile contained 37% trr. No further information was supplied on the hexane fraction. Silica gel chromatograhpy was used on the acetonitrile fraction to separate it into polar and nonpolar fractions. The non-polar fraction (32%

trr, 0.158 ppm) contained 7 metabolites (hplc), FNP1 to FNP7. FNP2 (8% trr, 0.042 ppm) was identified as 3-hydroxybutylmethyl sulfone by hplc, tlc, hplc, and gc/ms. FNP7 (7% trr, 0.061 ppm) was identified as tribufos by hplc, tlc, and gc/ms. FNP5 (5% trr, 0.024) was identified as n-butylmethyl sulfone by hplc.

Five metabolites ranging from <1% to 3% trr were found in the polar fraction (5% trr, 0.026 ppm). None could be identified. The major metabolite, FP1, 3% trr, eluted in the void volume (hplc, system B) and could be multiple metabolites. It was more polar than any of the standards (Figure 2).

The hexane fractions from the fat of ^{35}S-tribufos dosed chickens contained very little radiolabeled residue, about 2% compared with 20% for the ^{14}C-tribufos dosed chickens. The registrant speculates that the hexane fraction in ^{14}C-tribufos dosed chickens contained natural constituents (triglycerides, diglycerides, phospholipids) derived from butyric acid. No characterization is reported for the combined methanol/acetonitrile extract (83% trr, 0.255 ppm) of fat of ^{35}S-tribufos dosed chickens.

Muscle

The methanol extract (72% trr, 0.400 ppm) of muscle from ^{14}C-tribufos dosed chickens contained 9 compounds, MM-1 to MM-9, ranging from 1% trr to 29% trr (hplc, system A; tlc, system B). The major component, MM-4, 29% trr or 0.163 ppm was identified by tlc as 3-hydroxybutylmethyl sulfone. No confirmation is reported.

The ethyl acetate extract (24% trr) of the acid hydrolysis of the methanol extract contained (hplc, system B) 6 compounds, MMH01 to MMH06, ranging from <1% to 11% trr. Those compounds >1% trr were identified. MMH01, 11% trr or 0.060 ppm, was identified by hplc and hplc and gc/ms of the acetylated derivative as HBM sulfone. MMH05, 7% trr or 0.041 ppm, was identified by hplc and gc/ms as butylmethyl sulfone. MMH06, 3% trr or 0.016 ppm, was identified (hplc) as 3-hydroxybutylmethyl sulfone acetate. This, according to the registrant, is most likely the result of esterification by the extracting solvent.

The aqueous fraction (53% trr, 0.298 ppm) contained (hplc, system B) three metabolites, MMHA1 (20% trr, 0.109 ppm), MMHA2 (24% trr, 0.131 ppm), and MMHA3 (10% trr, 0.057 ppm). MMHA1 was identified as malic acid, COOH-CHOH-CH_2-COOH, through ion-pairing hplc and hplc of a methylated derivative. MMHA2 was identified as 3-hydroxybutylmethyl sulfone by hplc, tlc, and hplc and gc/ms of the acetylated derivative. MMHA3 was isolated and analyzed by gc/ms, but identification was not possible. No details are provided on this gc/ms analysis.

The methanol/water extract of muscle (85% trr, 0.432 ppm) from ^{35}S-

tribufos dosing of chickens contained at least 7 metabolites (hplc, system B), ranging from 3% trr to 31% trr. MSM5, 29% trr or 0.147 ppm, was tentatively identified (hplc) as 3-hydroxybutylmethyl sulfone. No further characterization was reported.

Eggs

Internal eggs (1.872 ppm) were used for metabolite investigation because the day 2 eggs contained only 0.281 ppm radiolabeled residue. The presence of extremely polar materials hindered the analysis of the methanol extract (39% trr, 0.733 ppm). The tlc (solvent system C) revealed radiolabeled compounds with R_f's similar to those of phospholipids (phosphatidyl choline, lysophosphatidyl choline, phosphatidyl ethanolamine, lysophosphatidyl choline). The tlc chromatograms were included. The hplc (system A or E) revealed at least 12 radiolabeled compounds, ranging from 0.2% trr to 8.3% trr. About 6.2% was unaccounted and was assumed to be retained by the column. Two major components, EMM5 and EMM10, each 6.1% trr, were isolated by isocratic hplc on a silica column. EMM5 was found to consist of two compounds, EMM5a and EMM5b.

The positive chemical ionization mass spectrum of EMM5a, a copy of which was included, was consistent with a diglyceride containing one linoleic (9,10-octadecadienoic, MW 280) and one oleic (9-octadecenoic, MW 282) fatty acid. Such a digylceride has a molecular weight of 618 (280 + 282 + 92 - 2X18) . The CI mass spectrum showed the requisite M + 1 and M + 20 peaks, 619 and 639. The EI mass spectrum of EMM5b, a copy of which was included, was consistent with a mixture of C-20 monoglycerides, MW 386 (saturated, $(CH_3)(CH_2)_{18}COOCH_2CH(OH)CH_2(OH)$) and 384 (one double bond).

The CI mass spectrum for EMM10 was unusually complex. The registrant concludes that the spectrum is consistent with a mixture of phosphatidyl cholines containing fatty acids of 16 and 18 carbons. A phosphatidyl choline containing a 16 carbon fatty acid and a 18 carbon fatty acid would have a molecular weight of 760, and m/z 761 (M + 1) is a major ion. Removal of the phosphatidyl side chain (m/z 183) yields m/z 577, and m/z 578 (M + 1) is the ion of greatest apparent abundance in the spectrum. This does not, however, explain minor ions 789, 813, 844, and 868.

The methanol fraction was transesterified with methanolic HCl, the mixture was diluted with water, and the mixture was partitioned sequentially with pentane and chloroform. The radiolabeled residue distributed as follows: pentane, 26% trr or 0.484 ppm; chloroform, 4% or 0.080 ppm; and water/methanol, 9% or 0.169 ppm. The hplc (system E) revealed four compounds, EMMP1 (20% trr, 0.373 ppm), EMMP2 (<1% trr, 0.010 ppm), EMMP3 (4% trr, 0.069 ppm), and EMMP4 (2% trr, 0.031 ppm). EMMP1 eluted (hplc) in a region consistent with a fatty acid methyl ester. EMMP3 eluted at the

retention time of tribufos. No additional characterization is reported. The hplc chromatogram (system E) of the chloroform extract revealed six compounds, as follows: EMMC1 (1% trr, 0.019 ppm), EMMC2 (<1% trr, 0.004 ppm), EMMC3 (1% trr 0.019 ppm), EMMC4 (<1% trr, 0.004 ppm), EMMC5 (<1% trr, 0.003 ppm), and EMMC6 (2% trr, 0.031 ppm). No additional characterization is reported.

The chloroform/methanol fraction (51% trr) from the initial extraction of internal eggs was shown to contain three compounds (hplc, system C), as follows: EFM1 (8% trr, 0.149 ppm), EFM2 (42% trr, 0.909 ppm), and EFM3 (<1% trr, 0.011 ppm). EFM2 was isolated via hplc and analyzed by positive chemical ionization mass spectrometry. The spectra of EFM2 and a triglyceride standard (triolein) were submitted. The spectrum is consistent with a mixture of three fatty acids, MW 855, MW 857, and MW 885. MW 885 is triolein, and the predominant peaks in the triolein standard are present in the unknown spectrum. MW 855 is consistent with a triglyceride containing one palmitoleic fatty acid ($CH_3(CH_2)_5CH=CH(CH_2)_7COOH$), one linoleic fatty acid ($CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$), and one oleic fatty acid ($CH_3(CH_2)_7CH=CH(CH_2)_7COOH$). MW 857 is consistent with a triglyceride containing one palmitic fatty acid (n-hexadecanoic), one linoleic fatty acid, and one oleic fatty acid. EFM2 and triolein had the same R_f values on silica tlc.

The assignment of triglyceride structures was confirmed by transesterification and analysis of the resulting methyl esters (FAME). The pentane extract contained 49% trr, 0.909 ppm. The one apparent compound (EFMP1, 49% trr) was isolated by hplc (system C, retention time about 6 min.). It had a R_f (tlc) comparable to those of methyl palmitate and methyl stearate. EFMP1 was analyzed by gc/ms which revealed a mixture of FAME's: methyl palmitoleate (MW 268), methyl palmitate (MW 270), methyl oleate (MW 296) methyl linoleate (MW 294), and methyl stearate (MW 298). Tribufos was absent.

Internal eggs from the ^{35}S-tribufos dosed birds were analyzed, because the residue levels were higher than in day 2 eggs, 0.848 ppm versus 0.352 ppm. The extraction profile was dramatically different from that of the ^{14}C-tribufos dosed birds (Table 5), and the registrant attributes the large amount of unextracted residue (70% trr) to incorporation of ^{35}S into calcium sulfate. The methanol extract (28% trr, 0.242 ppm) was analyzed by tlc. Most of the radioactivity (12% trr) failed to move off the origin, indicating inorganic sulfate or a sulfated natural product. In contrast to the ^{14}C-tribufos residues, little of the ^{35}S-residues could be extracted into chloroform/methanol (<1% trr, 0.003 ppm). No additional characterization was made.

Excreta

The hplc chromatogram (system A) of the methanol extract of excreta from ^{14}C-tribufos dosed chickens showed at least 18 components. Fractions were collected and purified by hplc. Tribufos, dibutyl disulfide (DBDS), malic acid (major metabolite), and 3-hydroxybutylmethyl sulfone (HBM sulfone) were identified by a variety of mass spectral techniques. Representative mass spectra were included.

Barium chloride precipitation of the methanol/water extract of excreta from ^{35}S-tribufos dosed chickens showed that 6% trr was inorganic ^{35}S-sulfate. The registrant claims that this 6% calculates to 3,511 μg of parent equivalent, and that that is more than the total amount of tribufos equivalents found in all collected tissues. No details or raw data are provided. The implication is that carbon-sulfur bond cleavage was the major path of degradation.

Five fractions were collected from the hplc (chromatogram not submitted) of the methanol/water extract of ^{35}S-tribufos dosed hens. One metabolite was isolated and identified by mass spectral analysis. That compound was identified as 3-hydroxy-butylthiophosphate. Spectra were submitted.

Proposed Metabolic Pathway

The registrant proposes the metabolic pathway shown in Figure 3, a copy of Figure 46, page 101, of MRID 42350010. Tribufos was extensively metabolized and was positively identified (<10% trr) only in fat. Tibufos is hydrolyzed to butyl mercaptan (BUSH) and S,S-dibutylphosphorodithioate (Dibufos). Dibufos (not found) was further hydrolyzed to bufos (not found) and ultimately to more BUSH and phosphoric acid. BUSH (not found) was further metabolized to butyric acid (not found) and subsequently incorporated into natural products, such as fatty acids, glycerides, and phospholipids. Butyric acid is a naturally occurring intermediate in the synthesis of fatty acids. Some BUSH may have been incorporated into proteins (containing S-S) and some BUSH was converted to 3-hydroxybutylmethyl sulfone (HBM sulfone), found in muscle, liver, fat, and excreta. Additionally, butylmethyl sulfone, a possible precursor of HBM sulfone, was found in fat, liver, and muscle. HBM sulfone could form sulfate and glucuronide conjugates. The presence of radiolabeled malic acid in liver and muscle suggests the breakdown of butyric acid to acetic acid and conversion of acetic acid to malic acid (Krebbs cycle).

Excreta was found to contain 3-hydroxybutylthiophosphate. Therefore, formation of HBM sulfone may have proceeded through a second path, hydroxylation of the butylthio group while still attached to the phosphate. Hydrolysis would yield 3-hydroxybutyl mercaptan, which would be converted to HBM sulfone via methylation and oxidation.

Nature of the Residue in Ruminants

In-Life Phase

The test substance was 1-^{14}C-tribufos, with the ^{14}C label on the CH_2 attached to the S of one of the butylthio groups. The specific activity was 20.4 mCi/mmol, and the radiochemical purity was >97.5%. No natural abundance tribufos was used in preparing the test formulation. The test doses were prepared in ethanol (52.97 μg/μl goat A and 66.34μ/μl goat B) and added to gelatin capsules containing lactose. The capsules were stored frozen until used. The ethanol solutions were analyzed by tlc and hplc.

Two lactating French Alpine goats, designated Goat A (34 kg) and Goat B (39 kg), were housed in steel cages and acclimated for four days prior to testing. The room was climate-controlled (25° C, 70% relative humidity) with 16 hours of light and 8 hours of dark each 24 hour period. Alfalfa hay and water were provided *ad libitum*. A dietary supplement (Purina) was provided at each milking. Milking was performed twice daily. Information on feed consumption (weight) was not provided. Therefore, published consumption figures were used to calculate the dose exaggeration rate (see below).

Each goat was dosed on 11/02/90 and subsequently at 24 hours and 48 hours. Each dose for Goat A contained 27.81 mg ^{14}C-tribufos (1.80 mCi), and each dose for Goat B contained 33.17 mg ^{14}C-tribufos (1.82 mCi). The dosing rates were 0.818 mg/kg body weight/day for Goat A and 0.850 mg/kg body weight/day for Goat B. Feces and urine were collected daily, and urine was radioassayed. Milk samples were radioassayed and stored frozen (-24° C). The goats were sacrificed 21 hours after the final dose. The following tissues were taken: liver, kidney, fat (composite), and muscle (composite). The tissues were processed into fine powders, radioassayed, and stored frozen.

Based on the 4 ppm tolerance for tribufos in/on cottonseed and the 6 ppm tolerance for tribufos in/on cottonseed hulls, on 15% of dairy or beef cattle diet being derived from cottonseed hulls and on 45% being derived from cottonseed or cottonseed processed commodities (Subdivision O, Table 2), and on 5 lbs. (2268 g) of feed per day per goat (*Feeds and Feeding,* Abridged 9th edition,Morrison, p. 458), the 0.818 ppm and 0.850 ppm doses represent 4.5X and 5.4X exaggerated feeding rates:

Theoretical Average Daily Dose-
{[(0.15) X (6 μg tribufos/g feed) + (0.45) X (4 μg tribufos/g feed)] X (2268 g feed/day/goat)} / 34000 g or 39000 g body weight = 0.180 μg tribufos/day/g body weight for Goat A and 0.157 μg/day/g body weight for Goat B.

Actual Dose Administered-
Goat A: 27.81 mg ^{14}C-tribufos/day/34000 g = 0.817 µg/day/g body weight. Equivalent to 12.3 ppm tribufos in the feed.
Goat B: 33.17 mg ^{14}C-tribufos/day/39000 g = 0.850 µg/day/g body weight. Equivalent to 14.6 ppm tribufos in the feed.

Exaggeration-
Goat A: 0.817/0.180 = 4.5
Goat B: 0.850/0.157 = 5.4

This contrasts with the registrant's calculation of a 25X exaggeration, based on a diet of 20% cottonseed and 5% hulls and a daily feed consumption of 3% of body weight (about 1100 g).

Analysis

Radioassay, Extraction, and Derivatization

Milk (70 ml, final day) was frozen and lyophilized. The lyophilized milk was extracted sequentially with ethyl acetate and methanol. The residual solids were mixed with water and acetone (1:2) and filtered.

Normal phase hplc of the ethyl acetate extract revealed four peaks, M1 (18% trr), M2 (4% trr), M3 (33% trr), and M4 (5% trr). The compounds were separated by preparative hplc. M1 was refluxed for 2 hours in 0.8N HCl/methanol (transesterification). The cooled mixture was neutralized and filtered, and the filtrate was partitioned with pentane/water (1/1). The pentane was evaporated, and the oily residue (M1F) was dissolved in hexane for hplc analysis. A portion of M1F was refluxed in 2 ml of 2N KOH/ethanol for 3 hours, cooled, and mixed with water. The mixture was partitioned with pentane. The aqueous layer was acidified and extracted with methylene chloride. The methylene chloride extract was dried over sodium sulfate, filtered, and evaporated to dryness. The residue was derivatized with O-p-nitrobenzyl-N,N'-diisopropylisourea (PNBDI) by reaction in methylene chloride for 3 hours at 85° to 90° C. The mixture was purified by preparative silica gel tlc. The scraped band was analyzed by reversed phase hplc.

A portion of the milk solids from the initial extraction (after suspension in water/acetone), were mixed in 1 N KOH for 3 hours. The pH of the solution was adjusted to 8 and EDTA and DTT (dithiothrietol) were added. The mixture was stirred for 18 hours at room temperature. The product mixture was extracted with chloroform.

A second milk extraction was performed about 5 months later to demonstrate storage stability. Lyophilized milk was extracted sequentially with ethyl acetate and methanol. No further work was conducted on the residual solids.

A sample of fat (30.0 g) was macerated sequentially with hexane and

acetonitrile. The dried residual solids were suspended in acetonitrile/water (1/1) and extracted with pentane. Centrifugation was needed to break the ensuing emulsion.

The residual solids were extracted by a procedure designed to remove lipids (G. M. Gray, *Methods in Enzymology*, 1969). The solids were macerated twice with cold chloroform/methanol (1:1). The combined extracts were partitioned against 0.1M KCl. The chloroform layer was dried over sodium sulfate, concentrated, and lyophilized. This lipid fraction was refluxed in methanol/0.8N HCl for two hours (transesterification). The solution neutralized, filtered, and extracted with pentane. The pentane was removed by rotary evaporation.

A portion of the fatty acid methyl ester fraction was hydrolyzed, drivatized (PNBDI), and purified as with the milk fatty acids.

A second extraction of fat was conducted about 5 months after the first extraction to demonstrate storage stability. The extraction procedure was duplicated through isolation of the fatty acids (chloroform extract).

A liver sample (102.7 g) was macerated with methanol (2X), and the combined extracts were concentrated and dissolved in water/acetonitrile (9/1).

In a second experiment, about 10 months after the initial extraction, liver (28.9 g) was macerated with methanol (2X) and filtered. A portion of the methanol extract was refluxed with strong HCl for 3 hours. The liver solids were solubilized by refluxing for 3 hours in 2N KOH. The mixture was cooled, and the pH was adjusted to 8. The mixture was reduced (18 hours, room temperature) with EDTA and DTT (dithiothrietol, mild disulfide reducing agent) and extracted with chloroform (3X). The pH was of the mixture was adjusted to 1 - 2, and the mixture was extracted with chloroform (8X). The first three extracts and the last eight extracts were each oxidized with MCPBA. The products were partitioned separately with water and methyl t-butyl ether (MTBE).

A separate liver sample was fractionated into soluble compounds (I), lipids (II), nucleic acids (III), and proteins (IV). The tissue was homogenized sequentially with 10% trichloroacetic acid (2X; I), 1% potassium acetate in ethanol (II), chloroform/ethanol (3:1, 2X; II), and 1M perchloric acid (III).

A portion of muscle tissue (99.6 g) was macerated with methanol (2X). The solid residue was solubilized by refluxing with 2N KOH for 2.5 hours. The mixture was extracted with chloroform (3X). The residual aqueous layer was adjusted to pH 1 - 2 and extracted with chloroform (5X). All chloroform extracts were combined.

The chloroform extract was oxidized with MCPA and partitioned with

The residual radioactivity in the post-extraction milk solids (26% trr, 0.031 ppm) was shown not to be butyl mercaptan bound via disulfide linkage to milk proteins. Base solubilization and DTT reduction did not release significant radioactivity. The registrant speculates that the radioactivity is incorporated into natural constituents.

About 5 months was required to conduct the characterizations and identifications. A second frozen sample of milk was extracted after the five month period. The total extractable radioactivity was 70%, compared to 68% trr in the original extractions. The distribution between ethyl acetate and methanol was markedly different, 26% trr in ethyl acetate and 44% in methanol for the final extraction versus 60% and 8% in the initial work. Separation of the stored milk into two fractions that could not be reunited may have contributed to this difference. Tribufos, HBM sulfone, and BM sulfone represented 5%, 30%, and 3% trr in the stored milk analysis, versus 5%, 33%, and 4% trr, respectively, in the original milk. The tribufos residues were stable in the frozen milk for up to five months.

Fat

The pentane extract contained one compound (F1), shown by normal phase hplc and tlc co-chromatography to be tribufos (31% trr, 0.06 ppm).

The acetonitrile/water extract (3% trr) contained one peak (FP1) by reversed phase hplc with a retention time of about 12 minutes. FP1 coeluted with HBM sulfone on tlc.

The hexane extract (27% trr, 0.051 ppm) was not investigated. Based on the poultry metabolism work, the registrant speculated that this extract contained natural constituents.

The chloroform/methanol extract (10% trr) was partitioned with 0.1 M KCl, yielding 8% trr (0.015 ppm) in the chloroform fraction and 2% trr in the water fraction. The chloroform fraction was transesterifed (HCl methanol, reflux), and the product mixture was partitioned with pentane. The pentane contained 7% trr (0.013 ppm). The aqueous fraction (1% trr) presumably contained glycerol. The pentane fraction was hydrolyzed (2N KOH ethanol, reflux) and converted to the p-nitrobenzyl esters. The organosoluble extract contained 4.2% trr (0.008 ppm). The hplc chromatogram (uv) contained 5 peaks corresponding to the p-nitrobenzyl esters of myristic acid, oleic acid, palmitic acid, and stearic acid. Fractions were collected and radioassayed. The histogram showed a major peak at 18 minutes absent in the uv chromatogram. The registrant speculates that the peak in the result of column contamination. Analysis by chemical ionization mass spectrometry of the isolated compounds identified stearic acid, oleic acid, palmitic acid, and myristic acid.

The fat extractions, characterizations, and identifications covered a five month period. A second sample of fat, stored frozen, was extracted at the end of the period. Tribufos (31% trr), HBM sulfone (6% trr), and BM sulfone (1% trr) were found. No BM sulfone was found in the initial extracts. The hexane extract contained 25% trr, compared to 27% in the initial extraction. The residual solid contained 25% trr, compared to 29% in the initial work. The radiolabeled residues were stable in the frozen fat for the five month period.

Liver

Extraction with acetonitrile, methanol/water (4/1), methylene chloride, water, ethyl acetate, and n-butanol were unsuccessful. Methanol extracted 10% - 12% trr (0.41 ppm). The reversed phase hplc of the concentrated methanol extract revealed approximately nine peaks (L1 - L9). Peak L9 corresponded in retention time to tribufos and/or dibutyl disulfide (DBDS), <1% trr. L1 was the major peak, 4% trr, 0.14 ppm. All other peaks were $\leq$ 0.01 ppm.

Acid hydrolysis of the methanol extract resulted in the loss of 25% trr. The hydrolysate was analyzed by reversed phase hplc. About nine peaks were present. LA2 (1% trr, 0.035 ppm) corresponded in retention time to HBM sulfone. This was confirmed by tlc co-chromatography.

Solubilization of the liver solids (88% - 90% trr) with 2N KOH and reduction with DTT resulted in the loss of 12% trr (0.41 ppm). Chloroform extracted 49% trr (1.69 ppm), and 29% trr (1.00 ppm) remained in the aqueous mixture. The chloroform residue was oxidized with MCPBA to minimize the loss of apparent volatiles. The resulting mixture was partitioned with MTBE (14% trr) and water (27% trr); 8% was lost. The water fraction contained butane sulfonic acid (15% trr, 0.52 ppm), as determined by reversed phase hplc, including co-chromatography. This was confirmed by tlc co-chromatography. The registrant speculates that the radioactivity lost was most likely butyl mercaptan. The maximum butyl mercaptan concentration would be 35% trr, or 1.10 ppm. The MTBE fraction was not characterized. The 29% trr in the aqueous fraction was not further analyzed.

Another sample of liver was fractionated into solubles, lipids, nucleic acids, and proteins, as indicated in Table 2. This characterization scheme is referenced in *S.E.P.: Metabolism in Food Animals: Qualitative Nature of the Residue* as a method for establishing that radioactive residues are natural constituents. However, this procedure is to be used after extractions and hydrolyses fail to release the radioactivity. Base hydrolysis did release 88% trr, and it would have been appropriate to pursue characterization/identification of the aqueous fraction (29% trr) after hydrolysis and the MTBE fraction (14% trr).

A second methanol extraction of liver frozen for nine months gave a hplc profile similar to that of the first extraction. Radiolabeled residues were stable in frozen liver for nine months.

Muscle

The reversed phase hplc chromatogram of the methanol extract (49% trr, 0.029 ppm) showed one major peak (MS1) at about 5 minutes, 44% trr, 0.026 ppm, and several very minor peaks. MS4 (<1% trr) was isolated and shown by tlc to be a mixture (about 50% each) of tribufos and dibutyl disulfide (DBDS).

MS1 was isolated and analyzed by thermospray lc/ms, but the structure was not obvious from the ion profile, major ion m/z 170. High resolution ms of MS1 showed that a 153 fragment was the result of the loss of $-NH_3$ and not the loss of -OH. This indicated that m/z 170 was not the molecular ion, but rather an adduct of MS1 with ammonium ions from the ammonium acetate buffer of the lc system. M/z 170 was a minor ion, presumably due to residual ammonia, when direct insertion chemical ionization was used with isobutane as the ionization gas. The major ion was m/z 153, suggesting a molecular weight of 152.

MS1 was oxidized with pyridinium dichromate (PDC). This gave a less polar compound that was analyzed by electron impact ms. The molecular ion was m/z 150, suggesting that a ketone had been formed by oxidization of an alcohol. A possible structure was x-hydroxybutylmethyl sulfone. MS1 was isolated from goat urine and analyzed by nmr, which indicated that the hydroxy group was located at C-3. Co-chromatography (hplc, tlc) indicated that MS1 from urine was MS1 from muscle. An authentic 3-hydroxybutylmethyl sulfone (HBM sulfone) was synthesized and analyzed by ms and nmr to confirm the structure assignment.

The muscle solids (51% trr, 0.031 ppm) were solubilized with aqueous 2N KOH and reduced with DTT. The residue was partitioned as organosoluble (24%, 0.014 ppm), water soluble (10%, 0.006 ppm), and volatiles (lost, 10%, 0.006 ppm). The organosoluble fraction was oxidized with MCPBA before any concentration steps. Nonetheless, 6% trr was lost. The remaining radioactivity was partitioned between MTBE (5%, 0.003 ppm) and water (13% trr, 0.008 ppm). The water fraction consisted of butane sulfonic acid (13% trr, 0.008 ppm), based on reversed phase hplc. Maximum butyl mercaptan concentration was 29% trr, or 0.02 ppm. The MTBE fraction and the residual aqueous mixture were not further analyzed.

To demonstrate storage stability, a second sample that had been frozen for ten months was extracted. About 53% trr, 0.033 ppm, was extracted, compared with 49% in the original extraction. The major component was HBM sulfone, 53% trr, compared with 44% trr in the

original extract. Tribufos and/or DBDS was/were <2% trr. The residue was stable in frozen muscle for ten months.

Kidney

The methanol extract (54% trr, 0.19 ppm) yielded a reversed phase chromatogram with two major, very polar peaks (KP1, 27% trr; KP2, 9% trr) and minor peaks (K3 - K9, each <2% trr, <0.007 ppm). Analysis of KP1 with Q5 ion pairing reagent gave one hplc peak, corresponding in retention time to HBM sulfone sulfate. KP1 was acid hydrolyzed and analyzed by hplc. The only product was HBM sulfone. KP1 was also isolated in larger quantities from urine and shown by thermospray lc/ms to be the sulfate conjugate of HBM sulfone.

KP2 was shown by hplc and tlc to be HBM sulfone. KP9 (<1% trr, <0.01 ppm) corresponded in retention time to tribufos and/or DBDS.

The kidney post-extraction solids (46% trr, 0.161 ppm) were solubilized in aqueous 2N KOH and reduced with DTT. The reaction product mixture was partitioned with chloroform. Chloroform contained 19% trr, the aqueous fraction contained 16% trr, and 11% trr was lost (volatiles). The chloroform fraction was oxidized with MCPBA and concentrated. The residue was distributed between water (6% trr, 0.021 ppm), MTBE (4% trr, 0.014 ppm), and loss (volatiles, 9%, 0.032 ppm). The water fraction was shown (hplc) to contain butane sulfonic acid (5%, 0.016 ppm). Total radioactivity attributed to butylmercaptan was 25% trr, 0.089 ppm. The residual aqueous phase and the MTBE fraction were not further analyzed.

A methanol extraction was conducted on a kidney sample stored frozen for eight months. The methanol extract contained 49% trr, compared with 54% trr in the initial extraction. The hplc profile of the methanol extract of the stored sample compared favorably with that of the initial methanol extract. The HBM sulfone sulfate conjugate was 26% trr, compared with 27% trr in the initial extract. The HBM sulfone was 9% trr, compared with 9% trr in the initial extract. Radiolabeled residues were stable in frozen kidney for eight months.

Urine

Urine from Day 1 was analyzed by reversed phase hplc. Seventeen peaks (U1 - U17) were observed in the presence of Q5 ion pairing agent. The major component U2 (22% trr) was identified as HBM sulfone (nmr, ms, comparison to synthetic standard). U4 (19% trr) was identified as the gluguronid conjugate of HBM sulfone by fab/ms. U7 (16% trr) was identified as the sulfate conjugate of HBM sulfone (thermospray lc/ms). Co-chromatography (hplc) with standards gave tentative identifications as bufose (U8, 4% trr), butyl-γ-glutamylcysteinylglycine disulfide (GSSBu, U10, 3% trr), N-acetylcysteinyl butane disulfide (NACSSBu, U13, 2% trr), and

dibufos (U16, 5% trr).

Proposed Metabolic Pathway

The registrant proposes a metabolic pathway identical to that proposed for poultry. See Figure 3. In addition to the information supplied with the proposed poultry metabolic pathway, the registrant notes that mechanisms have been proposed for the enzymatic S-dealkylation of mercaptans. BUSH would be converted to bytyryl aldehyde and butanol. These would be easily oxidized to butyric acid, a fatty acid of the endogenous metabolic cycle. Butyric acid could also be cleaved to acetic acid and incorporated into an array of natural constituents.

Dibufos and Bufos, intermediates in the transformation of Tribufos to BUSH were tentatively identified in urine, 5% trr and 4% trr, respectively.

Summary of Ruminant and Poultry Metabolisms

The metabolites identified in ruminant and poultry matrices are summarized in Table 7.

Storage Stability in Soil (MRID 42350008).

The registrant has presented a study on the stability of tribufos and dibutyldisulfide in frozen sandy loam soil samples. The stability of pesticides in soil is not under the purview of CBRS and will not be reviewed.

Waiver Requests for Animal Commodity Analytical Method(s) Study (171-4(d)) and for Animal Feeding Studies (171-4(j)). Request to Delete Tolerances for Eggs, Meat, and Milk. (MRID 42350012).

Miles Inc. argues that the nature of the residue studies were conducted at exaggerated rates and that residues were low. Therefore, residues are not anticipated at normal exposure (1X), and feeding studies are not needed. Because residues are not anticipated, the registrant argues that enforcement analytical methods are not needed for meat, milk, and eggs, and that the existing tolerances for milk and ruminant meat should be revoked. The registrant maintains that a 40 CFR §180.6(a)(3) situation exists.

The poultry study was conducted at a 190X exaggerated rate. The registrant calculated the rate as 167X but used the 6 ppm tolerance for tribufos residues in/on cottonseed hulls as the tolerance for meal and soapstock. The 4 ppm tolerance for tribufos residues in/on cottonseed is the appropriate value to use for the processed commodities in the absence of feed additive tolerances.

Table 7: Summary of Metabolites Found in Poultry and Ruminants from the Feeding of ^{14}C-Tribufos

Matrix	Compound or Component (% trr)										Identified/ Characterized (% trr)
	Tribufos	HBM Sulfone	HBM Sulfone Sulfate Conjugate	BM Sulfone	Malic Acid	Fatty Acids	Diglycerides/ Triglycerides/ Phosphatidyl Choline	DBDS	Butyl Mercaptan	Natural Products[1]	
Poultry (190X; 4 µg/g BW/day)											
Eggs (1.9 ppm)	4[2]					12	61			55	77
Muscle (0.56 ppm)	N/D	38		7	20					9	65
Liver (4.9 ppm)	N/D	10		2[2]	9				0	24	36
Fat (0.50 ppm)	7	8		5						38	58
Ruminant (5X; 0.83 µg/g BW/day)											
Milk (0.17 ppm)	5	33		4		12			0		54
Muscle (0.06 ppm)	0.5	44						0.5	29[3]		74
Liver (3.5 ppm)	<1[4]	1							35[5]		36[6]
Kidney (0.40 ppm)	<1[7]	9	27						25[8]		61
Fat (0.19 ppm)	31	3				7					41

[1] ^{13}C-tribufos concentration less the ^{35}S-tribufos concentration. Assumes S will not be incorporated into natural products.
[2] Not confirmed.
[3] 13% determined as BSA. Remainder assumed based on loss (volatilization).
[4] Not confirmed.
[5] 15% determined as BSA. Remainder assumed based on loss (volatilization).
[6] Additional characterization: 10% soluble, 8% lipid, 1% nucleic acid, 81% protein.
[7] Not confirmed. Could be partially or totally DBDS.
[8] 5% determined as BSA. Remainder assumed based on loss (volatilization).

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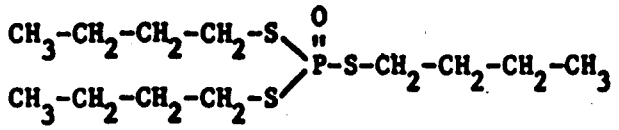
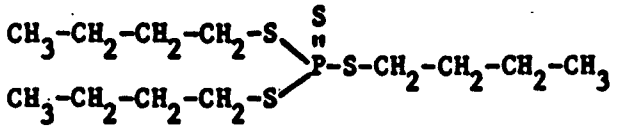
Regardless, the radiolabeled residues were < 0.6 ppm in fat and muscle. The residue was about 5 ppm in liver and about 2 ppm in the day 3 internal egg. The day 2 egg contained < 0.3 ppm trr. Residues did not plateau in eggs. Tribufos was found only in eggs (4% trr, 0.07 ppm) and fat (7% trr, 0.04 ppm). Clearly, tribufos would not be anticipated at or above detectable concentrations at 1X - 10X feeding rates. Given that tribufos is the only component of the residue of concern, a feeding study and enforcement analytical method are not required for poultry.

The goat metabolism study was conducted at a 5X exaggerated rate. The registrant calculated the rate as 26X, but used a 0 ppm level for tribufos in cottonseed meal and soapstock and a goat feed consumption that was too low. The trr may have plateaued at about 0.17 ppm in milk. The tissue residue levels ranged from 0.06 ppm in muscle to 3.4 ppm in liver. Given that the study was not conducted at a greatly exaggerated rate, significant residues would be anticipated in meat and milk at the 1X rate. Tribufos was 5% of the milk trr, or 0.008 ppm, and 31% of the fat trr, or 0.06 ppm. The current tolerances are set at 0.002 ppm for milk and 0.02 ppm for meat and fat. Such levels might be anticipated based solely on the results of this 5X goat metabolism study. Waivers of the ruminant feeding study and of the analytical method are not justified.

Figure 1: Names and Structures for Tribufos and the Analytical Standards Used in the Nature of the Residue in Cotton Study.



S,S,S-tributyl phosphorotrithioate



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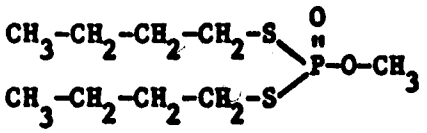
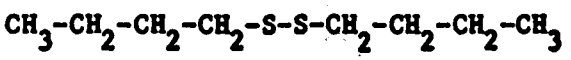
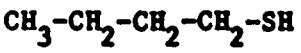


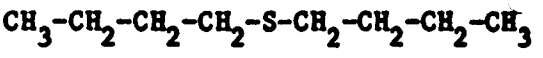
O-methyl *S,S*-dibutyl phosphorodithioate



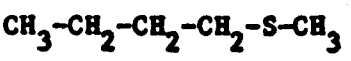
Dibutyl disulfide



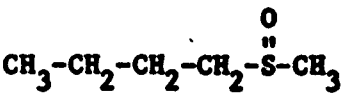
1-Butanethiol



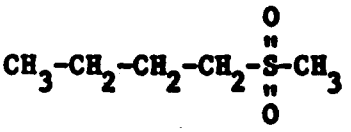
Butyl sulfide



n-Butyl methylsulfide



Methyl-*n*-butylsulfoxide



Methyl-*n*-butylsulfone

Figure 2: Names and Structures of Tribufos and Analytical Standards Used in the Nature of the Residue in Poultry and in Ruminants Studies.

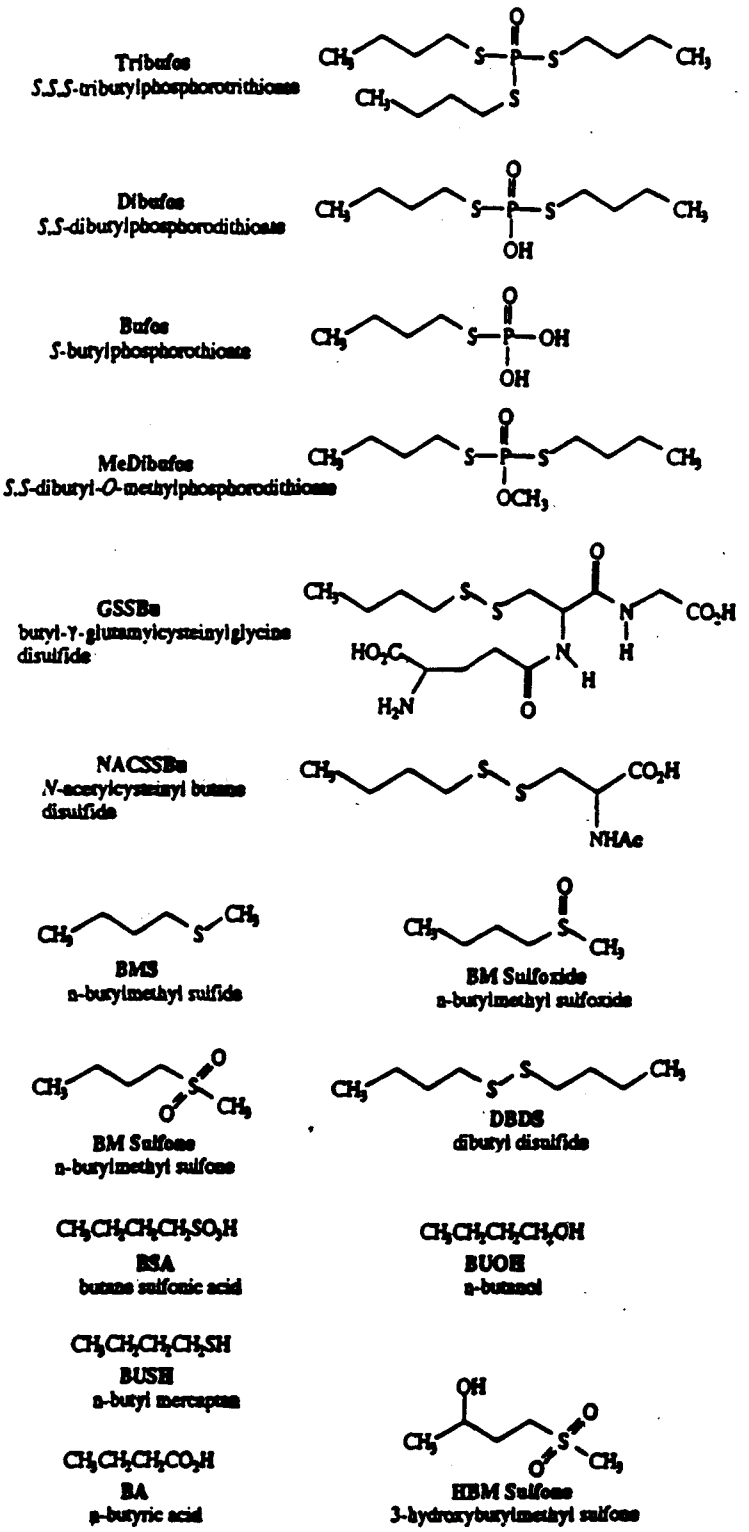
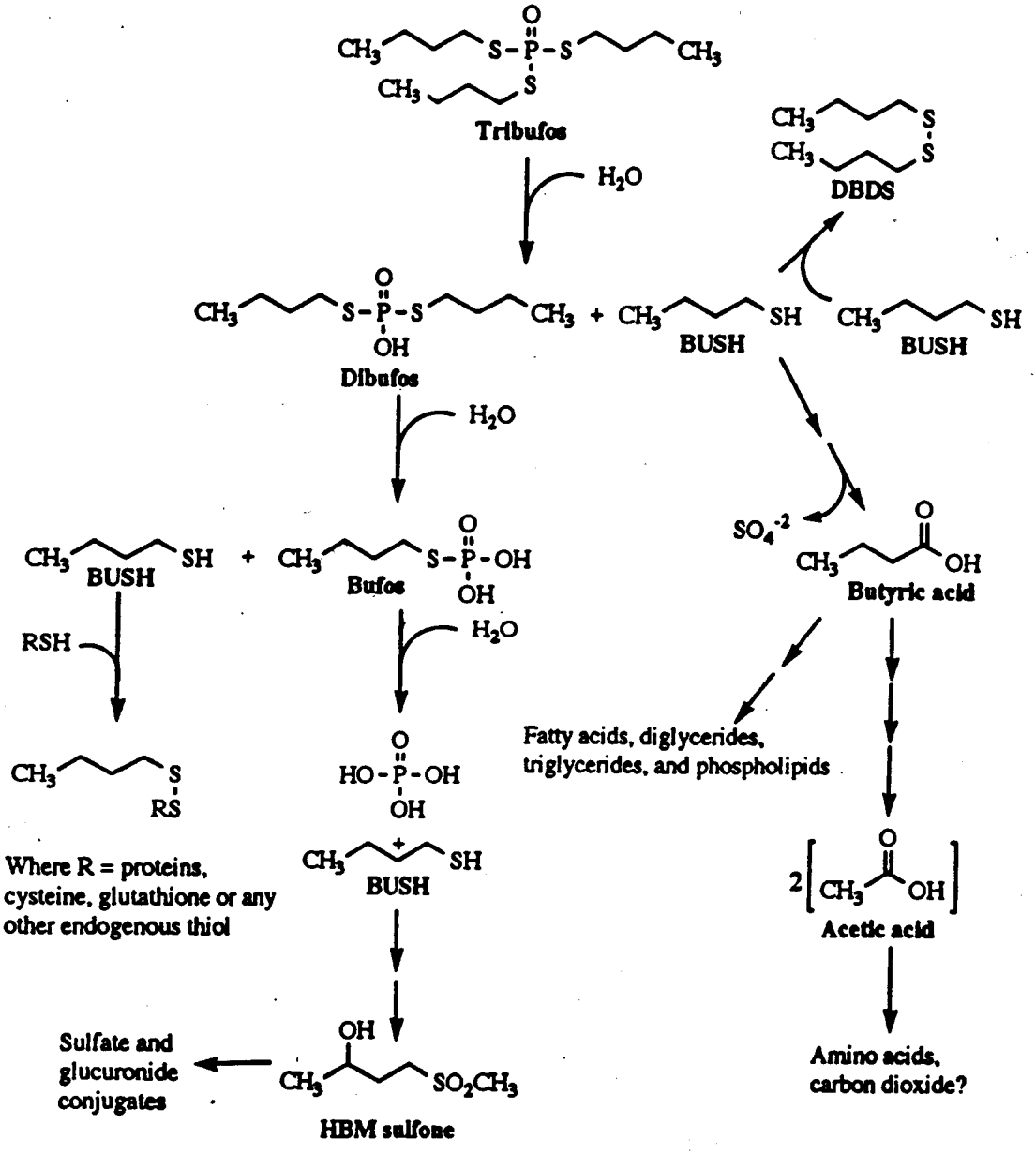


Figure 3: Proposed Pathway for the Metabolism of Tribufos in Laying Hens and in Goats.



cc: S. Funk, Tribufos List B File, SF, RF, Circ.

RDI:A. Rathman:11/19/93:M. Metzger:11/19/93:E. Zager:11/22/93:
H7509C:CBRS:S.Funk:305-5430:CM#2:RM803-A:SF(1093.12):11/18/93.

13544

R146245

Chemical: Tribuphos

PC Code: 074801
HED File Code: 61200 SRRD CDC
Memo Date: 11/23/1993
File ID: 00000000
Accession #: 000-00-8009

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